PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): Protocol for a randomised, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial.
AUTHORS	Lavender, Isobel; McCartney, Danielle; Marshall, Nathaniel; Suraev, Anastasia; Irwin, Chris; D'Rozario, Angela; Gordon, Christopher J.; Saini, Bandana; Grunstein, R; Yee, Brendon; McGregor, Iain; Hoyos, Camilla

VERSION 1 – REVIEW

REVIEWER	Walsh, Jennifer The University of Western Australia
REVIEW RETURNED	03-Feb-2023

GENERAL COMMENTS	Thank you for the opportunity to review this protocol for a randomised, placebo-controlled cross-over trial of two CBN doses on sleep and next-day function. It is clearly a very well-considered study design and well-written protocol by a reputable investigator team. It was a delight to read and I am eager to see the outcomes when the trial is complete. It is my opinion that high quality protocols like this should be published to provide a benchmark for future cannabinoid trials. I have made a few comments for consideration by the investigators although I appreciate that the trial is currently underway so some may not be useful for the present trial. Page 4 ln 29 - Being a single dose study without a familiarisation night it is also possible that there may be a first night effect, although the randomised design should account for this potential confounder Page 5 ln 48 - It would be helpful to provide a clearer explanation of the effects observed in the chronic dosing study. Given that the CBN decreased REM sleep initially following what is assumed to be a single dose, the reference to 'delayed effects' is a little confusing. Page 6 ln 3 – it would also be helpful to explain the sleep outcomes more fully - does the pre-clinical data suggest that total sleep duration is decreased soon after administration of CBN 10mg?

	Page 6 ln 8 – should be n=24 Page 6 ln 10 - it may be relevant to note that this outcome was assessed on night 14 of dosing Page 6 ln 37 - perhaps the definition of WASO should specify that it is wakefulness after sleep onset. ie. wakefulness during the time in bed period excluding the wakeful period prior to sleep onset Page 6 ln 31 - is there a reason for not using the most recent version of the scoring rules? Page 6 ln 37 - if possible it would also be good to get an indication of evening neurobehavioral function (to gauge intoxication or impairment) Page 8 ln 41 – I'm not sure if I've missed it but is there any contraceptive requirement for males in the trial? Page 8, ln 46 – again, not sure if I've overlooked it but those taking other sleep-promoting medications (eg. melatonin, z-drugs, OTC hypnotics) don't appear to be excluded Page 9 ln 22 - It is presumed that the IP is taken orally? Is there any instruction to withhold the oil in the mouth for a period of time? Page 10 ln 19 – the word 'physician' appears to be missing Page 10 ln 34 - should this be "A study physician may request to be unblinded in the event of a medical emergency"? Page 10 ln 48 – Is there an a priori definition of 'regular' sleep-wake schedule? If failing to achieve this, will participants be excluded? Page 11 ln 9 - the inclusion of standardised meals is a great addition which makes the PK data more robust and a clear sign that the investigator team have given this thorough consideration Page 11 ln 11 – have the authors considered repeating the ISI prior to each treatment arm? Page 11 ln 15 - has the IP been stability tested in this scenario (repeated extraction from the main storage vessel) over a period of 18months (anticipated trial duration)? Or will multiple storage vessels or batches be used? Page 13 ln 26 - To improve understanding of PK profiles it would be great to get plasma, urine and saliva samples at near simultaneous times relative to administration/time of day. It appears this is done at some times but not all. Page 13 ln 29 - 0700 urine sample is not shown in fig 2 Page 14 ln 18 - only 1 PVT is shown in fig 2 Page 14 ln 44 – It would be helpful to have more detail regarding EEG power and spindle analysis

	Page 15 ln 6 - it is assumed that the technician will be blinded to treatment?

REVIEWER	Rudisill, Toni West Virginia University, Epidemiology
REVIEW RETURNED	08-Feb-2023

GENERAL COMMENTS	This is a protocol for a randomized, double-blind, placebo-controlled, crossover, three-arm, proof-of-concept trial in 20 insomnia patients. The primary exposure will be cannabinol (CBN) at two doses (30 and 300 mg) vs. placebo. The primary outcome will be wake after sleep onset in minutes measured with polysomnography. Myriad of secondary and teritary outcomes will be investigated as well. This trial is important and necessary because so little is known about cannabinoids. Overall, the protocol is very clear and well thought. I only have two overarching comments. 1) I thought the statistical analysis section was a little weak. The authors mainly stated they were running linear mixed models. That is fine. However, I doubt that is the only statistics they will be completing. I felt there needed to be more detail about how the secondary and tertiary outcomes are being handled. Here is an example of what I am referring. The authors state they are using the PVT to measure vigilance—ok what aspect exactly? Are you using reaction time, number of errors, lapses in duration, etc? How are these going to be analyzed? That is just one example. Given the sheer amount of data they are collecting and analyzing, I felt more detail was needed. 2) I know the authors mentioned this but it still makes me uncomfortable. Many of the staff and the study are funded through the Lambert Initiative for Cannabinoid Therapeutics. I am always concerned about conflicts of interest. I am not sure anything can really be done about this.

REVIEWER	Pasipanodya, Jotam Praedicare Laboratories, Quantitative PreClinical, Clinical and Translation Department
REVIEW RETURNED	14-May-2023

GENERAL COMMENTS	Summary Medicinal and recreational use of tetrahydrocannabinols (THC) and cannabidiols (CBD), both primary and active pharmacological products contained in cannabis, has significantly increased with more widespread legislation of cannabis. There are clinical and pharmacokinetic (PK) studies suggesting both physiologic and pharmacologic interactions between THC and CBD; as well as, between either compound and other pharmaceutical drugs commonly used for medical and psychiatric indications. Other investigators have suggested inhibition of cytochrome enzymes as mediating the resulting adverse events; but there have not been enough PK studies of cannabinoids undertaken in ill patients [PMID 22716148; 2976654; 25316574]. Therefore, great caution is required prior to recommending THC and CBD for unrestricted clinical indications, especially for complex behavioral and habit-inducing conditions like insomnia. This reviewer is surprised that the CUPID study investigators did not include or were not explicit about a PK component for both efficacy and toxicity. The target pharmacodynamic space or organ

	is the brain, a physiologically protected physiologic space! There are several advantages for describing the population PK sub-study components, even for proof-of-concept trials, including identifying source of PK variability, better dose selection by linking exposures to different pharmacodynamic (PD) effects. In other words, population PD/PK models rather than statistical models are more appropriate means to examine and measures the impact of THC and CBD effect on sleep [as demonstrated in the paper cited by the investigators – see PMID 34115851]. The investigators already indicated on page 13 that they will measure blood, saliva, and urine levels of cannabinoids at different times post dose. Thus, the protocol would be better served by addressing the dose-concentration effect relationships, in addition to the primary and secondary outcomes already described.

VERSION 1 – AUTHOR RESPONSE

II. Reviewer 1 (Dr. Jennifer Walsh, The University of Western Australia)

Comments to the Author:

Thank you for the opportunity to review this protocol for a randomised, placebo-controlled cross-over trial of two CBN doses on sleep and next-day function. It is clearly a very well-considered study design and well-written protocol by a reputable investigator team. It was a delight to read and I am eager to see the outcomes when the trial is complete.

It is my opinion that high quality protocols like this should be published to provide a benchmark for future cannabinoid trials.

I have made a few comments for consideration by the investigators although I appreciate that the trial is currently underway so some may not be useful for the present trial.

1. **Page 4 ln 29 - Being a single dose study without a familiarisation night it is also possible that there may be a first night effect, although the randomised design should account for this potential confounder.**

CUPID participants are required to undergo a screening polysomnography (PSG) to determine eligibility (exclusion criteria #4). For screening PSGs, participants sleep in one of three identical rooms that are next-door to one another in a soundproofed enclave of the clinic. While the screening PSG serves as a familiarisation night, we acknowledge that some participants who have completed a diagnostic PSG at a separate clinic (in previous 12 months at doctor discretion) may be subject to a first night effect. We acknowledge that the randomised design (and potential prior experience with PSG setup) should account for this potential confounder.

2. **Page 5 ln 48 - It would be helpful to provide a clearer explanation of the effects observed in the chronic dosing study. Given that the CBN decreased REM sleep initially following what is assumed to be a single dose, the reference to 'delayed effects' is a little confusing.**

We anticipated this paper describing the preclinical acute and chronic CBN dosing study results would be published by the time we received reviewer comments on this manuscript, but unfortunately

submission has been delayed. As such, we have deleted the paragraph describing these results. We have made mention to the results regarding what is publicly available in the *32nd Annual Symposium on the Cannabinoids, International Cannabinoid Research Society* abstract (which can be viewed here: https://new.icrs.co/ICRS2022/ICRS2022/

3. **Page 6 ln 3 – it would also be helpful to explain the sleep outcomes more fully - does the pre-clinical data suggest that total sleep duration is decreased soon after administration of CBN 10mg?**

We agree and have now described the complete study outcomes (see Page 4, Line 27 and Box 1). As above, due to the limited public availability of preclinical CBN sleep results, we have changed the manuscript to only present the results described in the published abstract.

This abstract reports that acute administration of CBN (10, 30, and 100 mg i.p.) did not delay latency to NREM sleep in rats. They did identify a biphasic effect, however, with increased NREM sleep (in place of wake) and REM sleep percentage (with the 10mg/kg dose only) 4-hours post administration. Because it is difficult to compare preclinical and human sleep architecture, and CBN isolate has never been examined with respect to sleep architecture in humans, we did not necessarily hypothesise CBN effects on sleep architecture beyond a potential delayed effect which could reduce WASO.

We did however extend the period between drug administration and lights out to 2-hours (which was 1-hour in our previous study of cannabinoids on sleep in insomnia disorder [PMID: 32430450]).

4. **Page 6 ln 8 – should be n=24**

Thank you for pointing out this error. It has been fixed.

5. **Page 6 ln 10 - it may be relevant to note that this outcome was assessed on night 14 of dosing**

Agreed, this has been added.

6. **Page 6 ln 37 - perhaps the definition of WASO should specify that it is wakefulness after sleep onset. ie. wakefulness during the time in bed period excluding the wakeful period prior to sleep onset**

Agreed, we have updated WASO outcome as per clinical trial registry and clinical trial protocol (Page 4, Line 28): 'The primary study outcome is WASO minutes measured using in-laboratory overnight polysomnography, from the first epoch after lights out until the last epoch scored as any stage of sleep, scored by an experienced polysomnographic technician in accordance with American Academy of Sleep Medicine (AASM) 2020 Sleep Scoring criteria (Version 2.6).'

7. **Page 6 ln 31 - is there a reason for not using the most recent version of the scoring rules?**

The version was incorrectly reported as 2015. Scoring is according to the American Academy of Sleep Medicine (AASM) 2020 Sleep Scoring criteria (Version 2.6) which we have corrected. The latest update (Version 3.0, 2023) has not been implemented yet because it has not been reviewed by the Australia and New Zealand Sleep Science Association, ASA or ASTA.

8. **Page 6 ln 37 - if possible it would also be good to get an indication of evening neurobehavioral function (to gauge intoxication or impairment)**

We do include some brief measures of intoxication post drug administration (postural stability, subjective drug effects & a mood questionnaire). We agree that it would be interesting to ascertain evening neurobehavioral function as well. Due to the duration and intensity of study participation (up to 4 overnight sleep studies), we attempted to minimise testing to increase retention with a focus on next-day function. As this study investigates insomnia disorder and, in real-world application, patients would administer their dose just prior to sleep, we felt the more important question was next-day function.

9. **Page 8 ln 41 – I'm not sure if I've missed it but is there any contraceptive requirement for males in the trial?**

We instruct male and female participants to use contraception throughout study participation and to alert the research team immediately if they or their partner becomes pregnant and this is outlined in the PICF. All female study participants undergo a pregnancy test at the medical screening visit and prior to each of the three treatment sessions. We have now described this (Page 10, Line 1) adjusted the study eligibility criteria to clarify (Page 8, Line 12).

10. **Page 8, ln 46 – again, not sure if I've overlooked it but those taking other sleep-promoting medications (eg. melatonin, z-drugs, OTC hypnotics) don't appear to be excluded**

Our exclusion criteria stipulate use of CNS-active medications in the 3-months prior to treatment sessions (which includes most sleep medications such as melatonin, antihistamines, and z-drugs) or at sleep physician discretion. All concomitant medications and dietary supplements are documented at the medical screen (as well as at each treatment session and during follow up calls between them) and evaluated against the exclusion criteria.

11. **Page 9 ln 22 - It is presumed that the IP is taken orally? Is there any instruction to withhold the oil in the mouth for a period of time?**

The IP is in oral liquid form that is ingested via amber syringe. We do not instruct participants to hold the IP in their mouth. We instruct them to swallow it immediately and allow them to have a sip of water after, but they are not allowed to brush their teeth until 1.5 hours post administration. Because it is not a sublingual IP, we did not include a fixed period in the mouth but acknowledge there could be a risk of slightly different plasma concentrations because of this.

12. **Page 10 ln 19 – the word 'physician' appears to be missing**

Thank you for pointing to this error. It has been corrected.

13. **Page 10 ln 34 - should this be "A study physician may request to be unblinded in the event of a medical emergency"?**

We have updated this sentence for clarity.

14. **Page 10 ln 48 – Is there an a priori definition of 'regular' sleep-wake schedule? If failing to achieve this, will participants be excluded.**

Participants are asked to maintain a regular sleep schedule in the 7-night leadup, i.e., in bed between 10 and 11pm (whichever is closest to habitual sleep time). If there are minor deviations from the requested sleep schedule, such as sleep time 1-2 hours later/earlier on 1-2 nights, we proceed with the treatment session. If, on the other hand, participant sleep schedule is significantly irregular (i.e., > 2 hours on > 2 nights, napping throughout the day, or due to significant illness) the principal medical and non-medical investigators would determine whether the treatment session be rescheduled. This information has now been included in the manuscript (Page10, Line 36).

15. **Page 11 ln 9 - the inclusion of standardised meals is a great addition which makes the PK data more robust and a clear sign that the investigator team have given this thorough consideration**

Thank you.

16. **Page 11 ln 11 – have the authors considered repeating the ISI prior to each treatment arm?**

We deliberated over repeating the ISI at each visit during study design but decided it only be included as a measure of eligibility at the beginning of the study (baseline ISI is collected via the pre-screening questionnaire). We do, however, repeat the ISI prior to treatment session 1 (randomisation) if > 1.5 months has elapsed since the baseline ISI collection.

17. **Page 11 ln 15 - has the IP been stability tested in this scenario (repeated extraction from the main storage vessel) over a period of 18months (anticipated trial duration)? Or will multiple storage vessels or batches be used?**

The IP was manufactured, packaged, and labelled by the manufacturer (Medropharm, Switzerland) in early 2022. The IP is packaged in individual tincture bottles (3 per participant). Stability studies of the IP were conducted by the manufacturer in accordance with the International Conference on Harmonization (ICH) Guideline Q1A Stability Testing of new Drug Substances and Products and are ongoing. The CBN solution stability is 24 months (May 2024) at 15-25°C, 60% humidity and away from sunlight. The IP is stored in a temperature-controlled Schedule 9 safe in the Woolcock Institute Pharmacy. The anticipated study completion date is September 2023. This is now described in the manuscript (Page 9, Line 23).

18. **Page 13 ln 26 - To improve understanding of PK profiles it would be great to get plasma, urine and saliva samples at near simultaneous times relative to administration/time of day. It appears this is done at some times but not all.**

We agree that all biological samples (plasma, urine, and saliva) would ideally be collected at identical timepoints (baseline, 1.5-hours post, 11- hours post, and 13 hours post administration) and this was debated by investigators during study design. It was decided that blood collection prior and during sleep should be kept at a minimum as it may affect the primary outcome. Furthermore other restraints such as cost and feasibility limited the collection of other samples at all timepoints. The manuscript has been adjusted to mention the time of biological collection relative to drug administration.

19. **Page 13 ln 29 - 0700 urine sample is not shown in fig 2**

Thank you for pointing that out. Error corrected.

20. **Page 14 ln 18 - only 1 PVT is shown in fig 2**

Thank you for pointing this out, figure has been updated.

21. **Page 14 ln 44 – It would be helpful to have more detail regarding EEG power and spindle analysis**

We have now briefly summarised our procedure and referenced two papers which describe the methodology in greater detail (Page 15, Line 3): 'For quantitative EEG analyses, sleep polysomnography recordings will be processed using a previously validated automated artefact detection program. Power spectral and spindle analyses will derive from artefact-free epochs in central channels using previously published methodology.

22. **Page 15 ln 6 - it is assumed that the technician will be blinded to treatment?**

Yes, all study personnel and anyone in contact with participants or handling data are blinded. Only the Study Epidemiologist (NSM) and an independent staff member are aware of allocation order. We have now specifically mentioned the PSG scorer to be blinded (Page 15, Line 3).

III. Reviewer 2 (Dr. Toni Rudisill, West Virginia University)

Comments to the Author:
This is a protocol for a randomized, double-blind, placebo-controlled,
crossover, three-arm, proof-of-concept trial in 20 insomnia patients. The primary exposure will be cannabinol (CBN) at two doses (30 and 300 mg) vs. placebo. The primary outcome will be wake after sleep onset in minutes measured with polysomnography. Myriad of secondary and teritary outcomes will be investigated as well. This trial is important and necessary because so little is known about cannabinoids.
Overall, the protocol is very clear and well thought. I only have two overarching comments.

1. **1) I thought the statistical analysis section was a little weak. The authors mainly stated they were running linear mixed models. That is fine. However, I doubt that is the only statistics they will be completing. I felt there needed to be more detail about how the secondary and tertiary outcomes are being handled. Here is an example of what I am referring. The authors state they are using the PVT to measure vigilance—ok what aspect exactly? Are you using reaction time, number of errors, lapses in duration, etc? How are these going to be analyzed? That is just one example. Given the sheer amount of data they are collecting and analyzing, I felt more detail was needed.**

We thank Dr Rudisill for reviewing our paper and agree with his comments around the number of outcome measures. We are currently writing the Statistical Analysis Plan (SAP) detailing all analyses and statistical code which will be finalised prior to last patient last visit. This will be available upon request and/or published on an online database such as Research Gate. In the manuscript we have now described the full study outcomes (see Page 5, Line 27 and Box 1). We have also included more information about how measures will be analysed (see Page 17, Line 2).

2. **2) I know the authors mentioned this but it still makes me uncomfortable. Many of the staff and the study are funded through the Lambert Initiative for Cannabinoid Therapeutics. I am always concerned about conflicts of interest. I am not sure anything can really be done about this.**

The Lambert Initiative for Cannabinoid Therapeutics was established at the University of Sydney in 2015 from a philanthropic gift from Barry and Joy Lambert. The Lambert Initiative is a multidisciplinary research program within the Brain and Mind Centre at the University of Sydney with the primary aim of conducing the high-quality research required to discover, develop, and optimise safe and effective cannabinoid therapeutics in Australia and internationally. The Lambert Initiative activities extend from plant science and cannabinoid production, through cellular and preclinical pharmacology, to medicinal chemistry and drug discovery, including human laboratory studies and clinical trials; and act in an advocacy and educational capacity. Many researchers within the Lambert Initiative collaborate with external research groups. The funding of any project is overseen by a scientific committee known as the Internal Management Group (IMG) comprising of members external to the Lambert Initiative.

IV. Reviewer 3 (Dr. Jotam Pasipanodya, Praedicare Laboratories)

Comments to the Author:

Summary
Medicinal and recreational use of tetrahydrocannabinols (THC) and cannabidiols (CBD), both primary and active pharmacological products contained in cannabis, has significantly increased with more widespread legislation of cannabis. There are clinical and pharmacokinetic (PK) studies suggesting both physiologic and pharmacologic interactions between THC and CBD; as well as, between either compound and other pharmaceutical drugs commonly used for medical and psychiatric indications. Other investigators have suggested inhibition of cytochrome enzymes as mediating the resulting adverse events; but there have not been enough PK studies of cannabinoids undertaken in ill patients [PMID 22716148; 2976654; 25316574]. Therefore, great caution is required prior to recommending THC and CBD for unrestricted clinical indications, especially for complex behavioral and habit-inducing conditions like insomnia.

This reviewer is surprised that the CUPID study investigators did not include or were not explicit about a PK component for both efficacy and toxicity. The target pharmacodynamic space or organ is the brain, a physiologically protected physiologic space! There are several advantages for describing the population PK sub-study components, even for proof-of-concept trials, including identifying source of PK variability, better dose selection by linking exposures to different pharmacodynamic (PD) effects. In other words, population PD/PK models rather than statistical models are more appropriate means to examine and measures the impact of THC and CBD effect on sleep [as demonstrated in the paper cited by the investigators – see PMID 34115851]. The investigators already indicated on page 13 that they will measure blood, saliva, and urine levels of cannabinoids at different times post dose. Thus, the protocol would be better served by addressing the dose-concentration effect relationships, in addition to the primary and secondary outcomes already described.

We thank Dr Pasipanodya for taking the time to review the manuscript of our protocol publication. The current study aim is to investigate CBN effects on sleep in patients with insomnia disorder. The study aim was influenced by product marketing and widespread use of CBN for sleep in Europe and the United States despite an apparent absence of well-designed studies examining the effects of CBN isolate on objective and validated measures of subjective sleep. There exist some historical studies of CBN safety and toxicity (PMID: 6271848; PMID: 2960395; PMID: 6690168), some of which are described in a review of CBN and sleep (PMID: 34468204). Nevertheless, we echo the need for proper pharmacokinetic (PK) studies of CBN in humans.

During study design, we deliberated extensively over inclusion of PK components. Ideally, we planned to have four plasma collection timepoints during treatment sessions (baseline, 1 hour post drug

administration, upon wake, and pre-discharge); however, investigators firmly felt that measurements prior and during sleep could confound the primary outcome of WASO in an insomnia population. For this reason, we minimised blood collection to two time points during treatment sessions (baseline and upon wake). These data will serve as a manipulation check and will indicate CBN and metabolite persistence in plasma over a 13-hour period and between treatment sessions (which will also indicate carry-over effects). We agree that CBN PK data is of interest; however, it is beyond the scope of the current investigation which is focusing on an insomnia population.

Because of the low burden to participants, we were able to incorporate four urine collection timepoints across each treatment session which will provide some data around CBN metabolism at approximately 90-minutes (~21:30), 11-hours (~07:00), and 14-hours (~09:30) post drug administration. We expect further publications to arise from these data.

Reviewer: 1
Competing interests of Reviewer: I have no competing interests to report

Reviewer: 2
Competing interests of Reviewer: I have no competing interests.

Reviewer: 3
Competing interests of Reviewer: None

VERSION 2 – REVIEW

REVIEWER	Walsh, Jennifer The University of Western Australia
REVIEW RETURNED	10-Jul-2023

GENERAL COMMENTS	The authors have addressed my queries. I look forward to reading the outcomes of this study.

REVIEWER	Pasipanodya, Jotam Praedicare Laboratories, Quantitative PreClinical, Clinical and Translation Department
REVIEW RETURNED	24-Jul-2023

GENERAL COMMENTS	I concur with the other reviewers that the role of the funding agency should be clearly spelt out for transparency

VERSION 2 – AUTHOR RESPONSE

2. Reviewer: 1 Dr. Jennifer Walsh, The University of Western Australia

i. The authors have addressed my queries. I look forward to reading the outcomes of this study.

Thank you, Dr Walsh.

3. Reviewer: 2 Dr. Jotam Pasipanodya, Praedicare Laboratories

i. I concur with the other reviewers that the role of the funding agency should be clearly spelt out for transparency

Thank you Dr Pasipanodya. We have now provided a description of the funding agency, the Lambert Initiative for Cannabinoid Therapeutics, role.

Reviewer: 1
Competing interests of Reviewer: I have no competing interests

Reviewer: 3
Competing interests of Reviewer: None